

**WHAT IS CLAIMED IS:**

1                   1.       A charge-switch nucleotide phosphate (NP) probe, said NP probe  
2 comprising:

3                   an intact NP probe having a terminal phosphate with a fluorophore moiety  
4 attached thereto, said intact NP probe having a first molecular charge associated therewith,  
5 whereupon cleavage of said terminal phosphate as a phosphate fluorophore moiety, said  
6 phosphate fluorophore moiety carries a second molecular charge, wherein the difference  
7 between said first molecular charge and said second molecular charge is at least 0.5.

1                   2.       The charge-switch NP probe according to claim 1, wherein either said  
2 intact NP probe has a positive molecular charge, or wherein upon cleavage of said terminal  
3 phosphate fluorophore moiety, said terminal phosphate fluorophore moiety carries a  
4 molecular positive charge relative to said intact NP probe.

1                   3.       The charge-switch NP probe according to claim 1, wherein said  
2 charge-switch NP probe is a nucleotide triphosphate (NTP); and wherein said terminal  
3 phosphate is a pyrophosphate with a fluorophore moiety attached thereto.

1                   4.       The charge-switch NP probe according to claim 3, wherein said intact  
2 NTP probe has a positive charge.

1                   5.       The charge-switch NP probe according to claim 3, wherein upon  
2 cleavage of said terminal phosphate as a pyrophosphate fluorophore moiety, said  
3 pyrophosphate fluorophore moiety carries a positive charge relative to said intact NTP probe.

1                   6.       The charge-switch NP probe according to claim 3, wherein said NTP  
2 probe is a member selected from the group consisting of a deoxynucleotide triphosphate  
3 (dNTP), and a nucleotide triphosphate (NTP).

1                   7.       The charge-switch NP probe according to claim 6, wherein said NTP  
2 probe is a deoxynucleotide triphosphate (dNTP).

1                   8.       The charge-switch NP probe according to claim 7, wherein said  
2 deoxynucleotide triphosphate (dNTP) is a member selected from the group consisting of  
3 deoxyadenosine triphosphate, deoxycytosine triphosphate, deoxyguanosine triphosphate  
4 deoxythymidine triphosphate and deoxyuridine triphosphate.

1           9.       The charge-switch NP probe according to claim 6, wherein said  
2 nucleotide triphosphate (NTP) is a member selected from the group consisting of adenosine  
3 triphosphate, cytosine triphosphate, guanosine triphosphate and uridine triphosphate.

1           10.      The charge-switch NP probe according to claim 1, wherein said  
2 fluorophore moiety is a member selected from the group consisting of fluorescein, 5-  
3 carboxyfluorescein (FAM), rhodamine, 5-(2'-aminoethyl) aminonaphthalene-1-sulfonic acid  
4 (EDANS), anthranilamide, coumarin, terbium chelate derivatives, Reactive Red 4, BODIPY  
5 dyes and cyanine dyes.

1           11.      The charge-switch NP probe according to claim 3, wherein said  
2 fluorophore moiety is attached to said terminal phosphate via a linker.

1           12.      The charge-switch NP probe according to claim 11, wherein said  
2 fluorophore linker is an alkylene group having between about 5 to about 12 carbons.

1           13.      The charge-switch NP probe according to claim 11, wherein said linker  
2 carries at least one positive charge.

1           14.      The charge-switch NP probe according to claim 11, wherein said linker  
2 carries at least two positive charges.

1           15.      The charge-switch NP probe according to claim 1, wherein at least one  
2 of the phosphate moieties of said nucleotide phosphate probe has an ionized oxygen atom  
3 with a counter-cation associated therewith.

1           16.      The charge-switch NP probe according to claim 15, wherein said  
2 counter-cation is a metal ion.

1           17.      The charge-switch NP probe according to claim 16, wherein said metal  
2 ion is selected from the group consisting of  $Mg^{++}$ ,  $Mn^{++}$ ,  $K^{+}$  and  $Na^{+}$ .

1           18.      The charge-switch NP probe according to claim 11, wherein said  
2 fluorophore moiety is BODIPY TR.

1           19.      The charge-switch NP probe according to claim 1, wherein the  
2 difference between said first molecular charge and said second molecular charge is selected

3 from the group consisting of 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9,  
4 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, and  
5 4.0.

1           **20.**     The charge-switch NP probe according to claim 1, wherein said  
2 difference between the first molecular charge and the second molecular charge is calculated  
3 in pure water at pH 7.0.

1           **21.**     The charge-switch NP probe according to claim 1, wherein said  
2 charge-switch probe is selected from the group consisting of compound 50, 51, 52, 53, 54,  
3 55, 56, 57, 58, 59 and 60 in Figures 6A-D.

1           **22.**     A method for separating a labeled nucleotide phosphate having a  
2 detectable moiety from a released charged detectable moiety in a sample stream, said method  
3 comprising:

4           a)       immobilizing a complex comprising a nucleic acid polymerase or a  
5 target nucleic acid onto a solid support in a single molecule configuration;

6           b)       contacting said complex with a sample stream comprising a target  
7 nucleic acid when said polymerase is immobilized or a polymerase when said target nucleic  
8 acid is immobilized, a primer nucleic acid which complements a region of said target nucleic  
9 acid; and a labeled nucleotide phosphate having a detectable moiety, wherein said detectable  
10 moiety is released as a charged detectable moiety when said NP is incorporated into said  
11 primer nucleic acid; and

12           c)       applying an energy field to said sample stream, thereby separating said  
13 labeled NP from said charged detectable moiety.

1           **23.**     The method according to claim 22, wherein said labeled nucleotide  
2 phosphate (NP) is a labeled nucleotide triphosphate (NTP).

1           **24.**     The method according to claim 23, wherein said labeled nucleotide  
2 triphosphate (NTP) having a detectable moiety is a NTP having a  $\gamma$ -phosphate with a  
3 detectable moiety attached thereto.

1           **25.**     The method according to claim 23, wherein said charged detectable  
2 moiety when released comprises a pyrophosphate with a fluorophore moiety attached thereto.

1           **26.**     The method according to claim **24**, wherein said labeled NTP is  
2 incorporated into said nucleic acid primer hybridized to said target nucleic acid using said  
3 polymerase, thereby releasing said  $\gamma$ -phosphate with said detectable moiety attached thereto.

1           **27.**     The method according to claim **26**, wherein said target nucleic acid  
2 comprises a self-complementary region forming said primer.

1           **28.**     The method according to claim **22**, wherein the charge of said  
2 detectable moiety after release is different than said labeled nucleotide phosphate (NP)  
3 having a detectable moiety attached thereto.

1           **29.**     The method according to claim **28**, wherein the charge of said  
2 detectable moiety is more positive than the unincorporated labeled NP.

1           **30.**     The method according to claim **28**, wherein the charge of said  
2 detectable moiety attached thereto is opposite in sign compared to the unincorporated  
3 fluorescently labeled NP.

1           **31.**     The method according to claim **22**, further comprising  
2 d) measuring said detectable moiety with a measuring device.

1           **32.**     The method according to claim **31**, wherein said measuring device is  
2 selected from the group consisting of a charge coupled device (CCD) camera, a photodiode, a  
3 video chip, amp meter, voltage meter, and a dye-impregnated polymeric coating on optical  
4 fiber sensor.

1           **33.**     The method according to claim **32**, wherein said detection is via a  
2 CCD camera.

1           **34.**     The method according to claim **32**, wherein said detection is via a  
2 photodiode.

1           **35.**     An analytical method for separating an intact NP probe from a  
2 phosphate detectable moiety, said method comprising:  
3           a)     providing a sample comprising an intact NP probe with a detectable  
4 moiety attached thereto, whereupon enzymatic cleavage of said intact NP probe, which

5 produces a phosphate detectable moiety, said phosphate detectable moiety carries a molecular  
6 charge which is different than the molecular charge of said intact NP probe; and

7 b) applying an energy field to said sample, thereby separating said  
8 phosphate detectable moiety from said intact NP probe.

1 36. The method according to claim 35, wherein said NP probe with a  
2 detectable moiety is a labeled nucleotide triphosphate (NTP).

1 37. A method for sequencing a target nucleic acid with a polymerase, said  
2 method comprising:

3 a) immobilizing a complex comprising a nucleic acid polymerase or a  
4 target nucleic acid onto a solid support in a single molecule configuration;

5 b) contacting said complex with a sample stream comprising a target  
6 nucleic acid when said polymerase is immobilized or a polymerase when said target nucleic  
7 acid is immobilized, a primer nucleic acid which complements a region of said target nucleic  
8 acid of the region to be sequenced; and a labeled nucleotide phosphate having a detectable  
9 moiety, wherein said detectable moiety is released as a charged detectable moiety when said  
10 NP is incorporated into said primer nucleic acid wherein said solid support is attached to a  
11 flowcell having an inlet port and an outlet port;

12 c) applying an energy field to said sample stream; and

13 d) detecting said charged detectable moiety, thereby sequencing said  
14 target nucleic acid.

1 <sup>38</sup>~~40~~. The method according to claim 37, wherein said detectable nucleotide  
2 phosphate is a labeled nucleotide triphosphate (NTP) having a  $\gamma$ -phosphate with a detectable  
3 moiety attached thereto.

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1 ~~39~~<sup>41</sup>. The method according to claim 37, wherein said NP is incorporated on  
2 said primer strand hybridized to said target nucleic acid using said polymerase and thereby  
3 releasing said  $\gamma$ -phosphate with said detectable moiety attached thereto.

1 ~~40~~<sup>42</sup>. The method according to claim 37, wherein said energy field is an  
2 electric field.

1 ~~41~~<sup>43</sup>. The method according to claim ~~42~~<sup>40</sup>, wherein said electric field is a first  
2 electric field applied in the transverse direction and a second electric field applied in the axial  
3 direction.

1 ~~42~~<sup>44</sup>. The method according to claim 37, wherein the charge of said  $\gamma$ -  
2 phosphate with said fluorophore moiety attached thereto is greater than the unincorporated  
3 fluorescently labeled NTP.

1 ~~43~~<sup>45</sup>. The method according to claim 37, wherein the charge of said  $\gamma$ -  
2 phosphate with said fluorophore moiety attached thereto is less than the unincorporated  
3 fluorescently labeled NTP.

1 ~~44~~<sup>46</sup>. The method according to claim 37, wherein the charge of said  $\gamma$ -  
2 phosphate with said fluorophore moiety attached thereto is opposite in sign compared to the  
3 unincorporated fluorescently labeled NTP.

1 ~~45~~<sup>47</sup>. The method according to claim 37, wherein said detection is via a  
2 charge coupled device (CCD) camera.

1 ~~46~~<sup>48</sup>. The method according to claim 37, wherein said detection is via a dye-  
2 impregnated polymeric coating on optical fiber sensor.

1 ~~47~~<sup>49</sup>. The method according to claim 37, wherein said detection is via a  
2 blockade current.

1 ~~48~~<sup>50</sup>. The method according to claim 37, wherein said detection is via a  
2 photodiode.